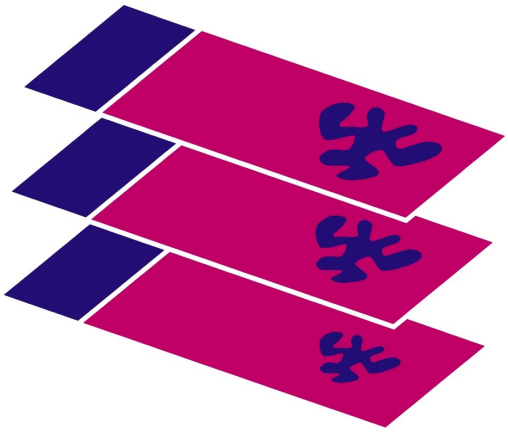

3DHISTECH

FISHQuant

Module 1.12

User's Guide

June 11, 2009

Contents

Disclaimer....3
Font types and symbols....4
Terms and abbreviations....5
1 About FISH Analysis....6
1.1 FISH Analysis in the MIRAX product family....6
1.2 Overview of FISH Analysis....7
1.2.1 FISHQuant algorithms....7
1.2.2 MISP files....7
1.2.3 Using a MISP file on other slides....7
2 Installing MIRAX Viewer and FISHQuant....9
3 Working with FISH Analyzer....10
3.1 Before you start....10
3.2 Creating an annotation for FISHQuant....10
3.3 Running the Algorithm on a Single Slide....11
3.4 Running Batch Process in FISHQuant....15
4 Working with Measurement Visualization....16
4.1 Creating a Histogram....16
4.2 Creating a Scatterplot....18
4.3 Creating a Gallery....19
4.4 Creating a Classification Gallery....21
Related documents....23
Index....24
Appendix A FISH Analysis....26
A.1 Object-specific parameters....26
A.2 Summing parameters for an area....26

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Font types and symbols

Example	Abbreviation or term that is explained in the section Terms and Abbreviations.
Example	Words or characters that appear on the screen. These include field names, screen titles, pushbuttons and menu names, paths or options. Keys on the keyboard. For example, function keys (such as **F11**) or the **Ctrl+O** key combination.
Example	Cross-references to other documents.
Warning!	Indicates that you need an additional module to use the feature or that there are prerequisites for the task or that special care should be taken when using certain feature of the device.
Important!	An important piece of information or a recommendation. The application will work if you choose not to follow the recommendation but its performance might be less than optimal.
Tip!	A suggestion about using the application in some other way or to some interesting purpose.

Terms and abbreviations

Annotation	A particular detail of a digitized slide. FISHQuant can perform measurements on annotations.
CSV format	Comma separated value
Digital slide	Slide digitized with a MIRAX scanner microscope, for example MIRAX SCAN. Consists of an MRXS file and a folder with the same name as the MRXS file.
Field	In image analysis module: an annotation. In ImmunoScreener: a grid cell.
Layer	A group of cell compartments matching the same stain and size criteria.
Measurement	FISHQuant can perform two types of measurements: • field-specific (related to the annotation) • object-specific
MISP	MIRAX Imaging Segmentation Profile An image analysis segmentation and measuring algorithm is stored in a MISP file.
MRXS	MIRAX Slide File extension of a digitized MIRAX slide.
Object	A cell compartment (for example the FISH), cell or tissue that is relevant for the immunohistochemical analysis.
Segmentation	Identification of cells or cell compartments based on their different reaction to immunohistochemical staining.

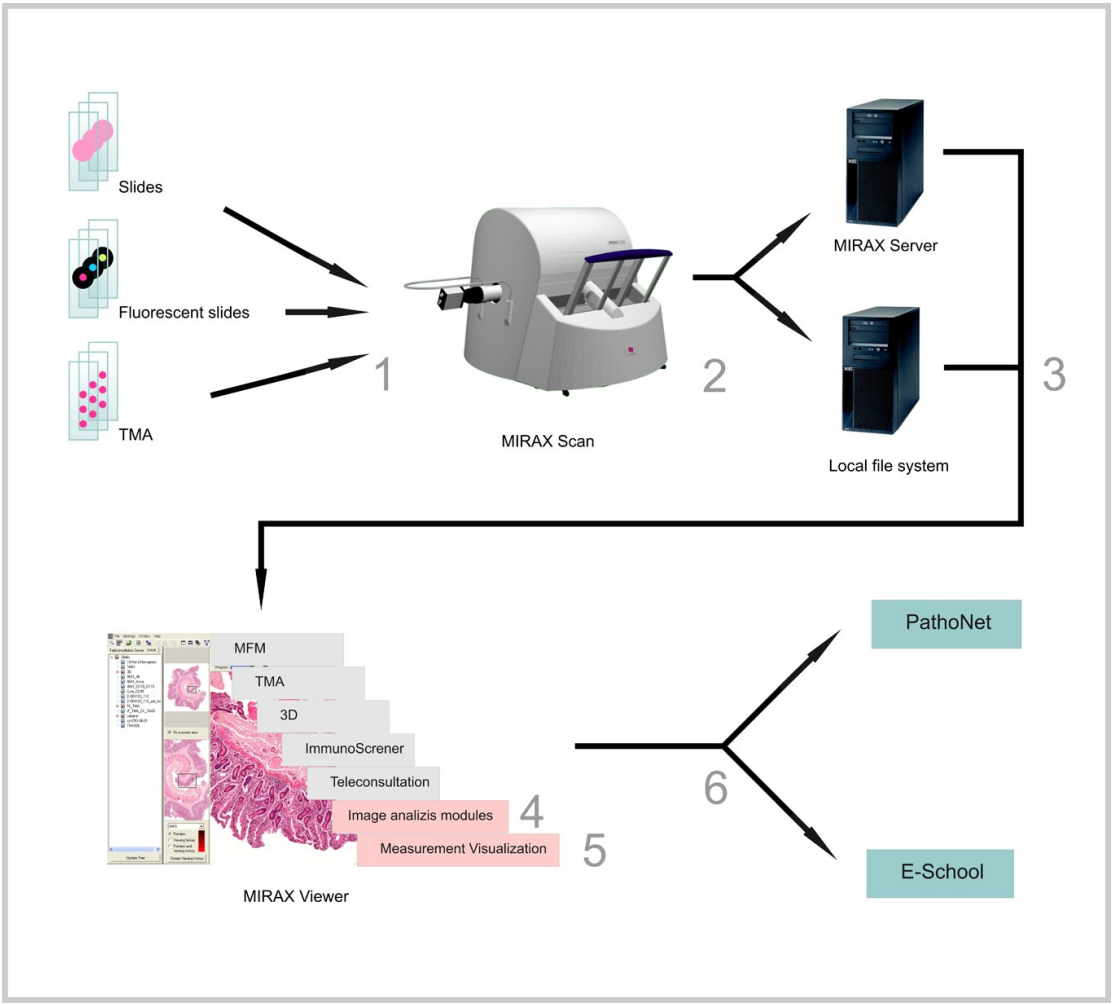
1 About FISH Analysis

FISHQuant is an optional module for MIRAX Viewer. It allows you to detect and separate FISH spots on the virtual slides and digitalized with MIRAX microscopes.

- FISH Analysis performs *segmentation* and *measurements* on MIRAX *digital slides* based on the colors of the spots.
- Based on the applied FISH probe it can classify the cell nuclei.

1.1 FISH Analysis in the MIRAX product family

The following picture shows how FISH and **Measurement Visualization** extend MIRAX Viewer and how they fit into the MIRAX product family.



1. Slides are digitized with MIRAX microscopes. A digitized slide consists of an *MRXS* file and a folder with the same name as the *MRXS* file.

2. Digitized slides are stored on a computer. You can also use a dedicated server (MIRAX Server) for storing the MIRAX slides.

3. Slides digitized with a MIRAX microscope can be viewed in MIRAX Viewer. If the slide is stored on a MIRAX Server, it is opened through the Teleconsultation module. Local slides are opened directly with MIRAX Viewer.

4. **FISHQuant** allows you to perform *measurement*s on *digital slide*s and to export the *measurement*s in *CSV format*.

5. **Measurement Visualization** displays the **FISHQuant** data as a scatterplot, a histogram or a gallery.

6. *Digital slide*s, including slides that store **FISHQuant** *measurement*s, can be shared via PathoNet. They can also be used in E-School training and exam material.

1.2 Overview of FISH Analysis

You can perform three types of tasks in **FISHQuant**:

- create algorithms based on your parameters and then run them on a slide or a set of slides

- view the *measurement* results with the **Measurement Visualization** module, save the *measurement annotation*s with the slide or export the *measurement* results to *CSV* file(s)

- create a report based your saved *measurement*s.

1.2.1 FISHQuant algorithms

FISHQuant allows you a FISH analysis setup application, which has a *segmentation* and a thresholding setup section. You can also change the score outlining color. The information needed for the algorithm is not saved on the slide but in a MIRAX Image Segmentation Profile (*MISP*) file, which can be used later to evaluate other *annotation*s on the same slide or on other slides.

1.2.2 MISP files

FISHQuant algorithms are saved in *MISP* files that store the following information about the algorithm:

- *segmentation* algorithm
  (filters for noise reduction, *segmentation* and *object* separation)

- classifications

1.2.3 Using a MISP file on other slides

You can reuse the *MISP* on other slides from the same batch (on slides that are from the same specimen) without modifications. You can also use the same algorithm on slides from other specimens with the same staining.

You might need to adjust the profile to ensure that differences in the specimen or stain quality do not affect the result.

2 Installing MIRAX Viewer and FISHQuant

1. Start the MIRAX Viewer **setup.exe** file that you received on a MIRAX Viewer DVD or downloaded in a compressed file.

2. Follow the instructions in the installation wizard.

3. Start the application **setup.exe** file that you received on the application DVD or downloaded in a compressed file.

4. Follow the instructions in the installation wizard.

3 Working with FISH Analyzer

3.1 Before you start

FISHQuant works on selected *annotation*s. You will be able to run other algorithms on several *annotation*s by selecting all the *annotation*s that you need when you start the algorithm.

Important!

FISH Analysis algorithms require a significant amount of computer resources. The resource need and the speed of the Membrane Analysis evaluation depends on the size and the number of the measured *annotation*s, and the quantity of detected *object*s.

To ensure accurate results and to reduce computation time:

- Make the training *annotation* as small as possible. The annotated areas should not be larger than 300x300 µm (1:1 magnification).
- When you are creating a free-hand *annotation*, follow the outline of the area as closely as possible.
- Membrane Analysis only works on slides to which you have write access. You may have only read permission to a slide if you access it through Teleconsultation and the host only gave you read permission, or if you access a slide stored on a network drive and you only have read, but not write permission to the drive.

Tip!

If you need *measurement*s on the entire specimen, put an *annotation* on the slide to cover the entire specimen.

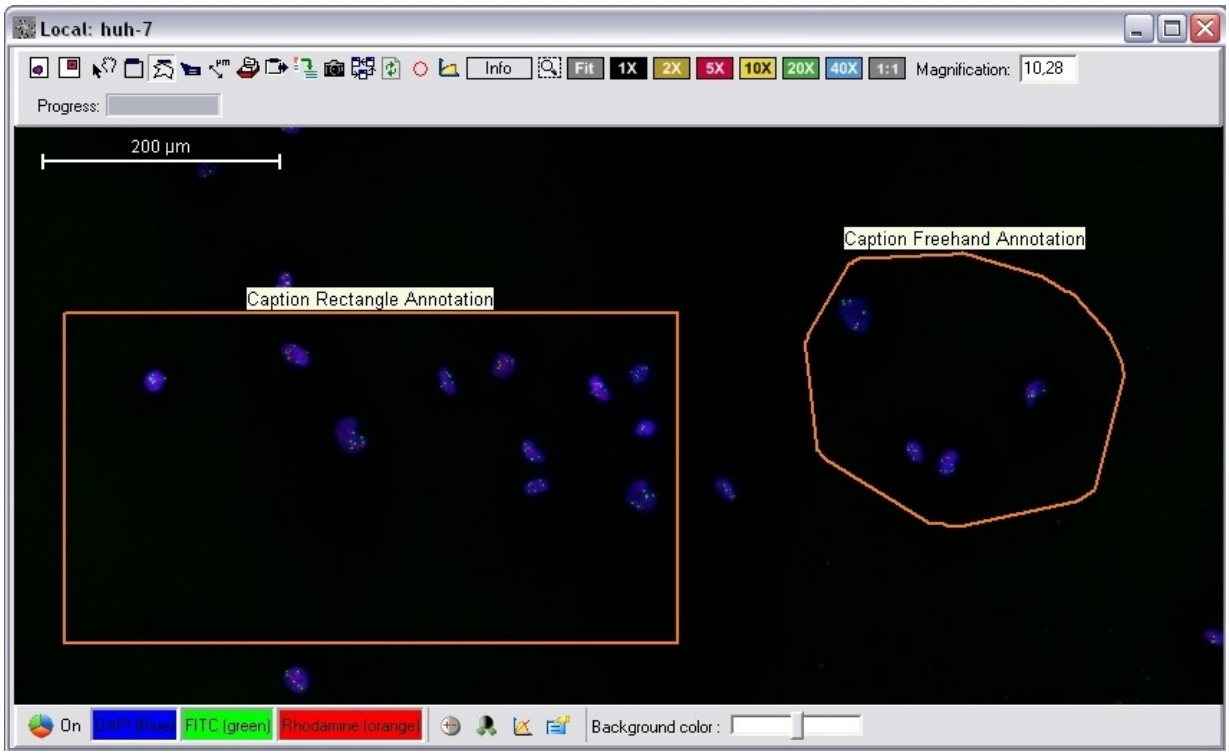
3.2 Creating an annotation for FISHQuant

1. Open the slide in MIRAX Viewer. For more information on how to open slides in MIRAX Viewer, see section **Working with slides in MIRAX Viewer** in the *MIRAX Viewer 1.12 User's Guide*.
2. Create the *annotation* on the area of your interest:
   - Select the *annotation* type (rectangle or free-hand) and cover the area with the *annotation*.

For information on navigating and zooming on the slide, see section **Viewing slides** in document *Mirax Viewer 1.12 User's Manual*.

- In the **Create or Edit Annotation** window add a title to the *annotation*, enter a comment and determine the background color of the title and the outline color of the *annotation*. You can either select from predetermined colors or you can define custom colors.

  Only captions are exported to the *CSV* output file. *Annotation* descriptions are visible only in MIRAX Viewer.



3. Repeat step 2 for each area on which you want to run the algorithm.

3.3 Running the Algorithm on a Single Slide

Open the slide in MIRAX Viewer.

1. In the main toolbar, click and select **FISHQuant** from the **Image Analysis Modules**.

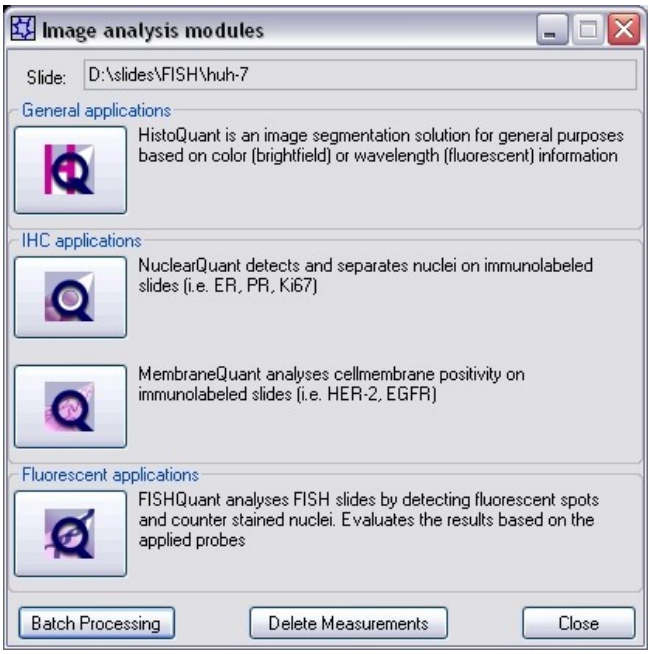


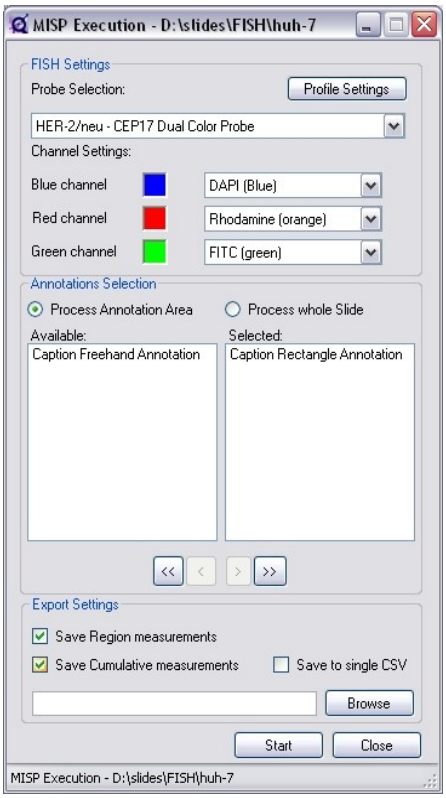
Image analysis modules
Slide:
D:\slides\FISH\huh-7
General applications
HistoQuant is an image segmentation solution for general purposes based on color (brightfield) or wavelength (fluorescent) information
IHC applications
NuclearQuant detects and separates nuclei on immunolabeled slides (i.e. ER, PR, Ki67)
MembraneQuant analyses cellmembrane positivity on immunolabeled slides (i.e. HER-2, EGFR)
Fluorescent applications
FISHQuant analyses FISH slides by detecting fluorescent spots and counter stained nuclei. Evaluates the results based on the applied probes
Batch Processing
Delete Measurements
Close

2. Select a **FISH probe** from the roll-down list.

3. If it's not automatical, choose the adequate channel name for the colors.

4. Select the *annotation(s)* you want to analyze in the **Available** window and move them to the **Selected** window. Also the whole slide can be selected to work with.

5. Select **Profile Settings** to define the parameters of the algorithm.

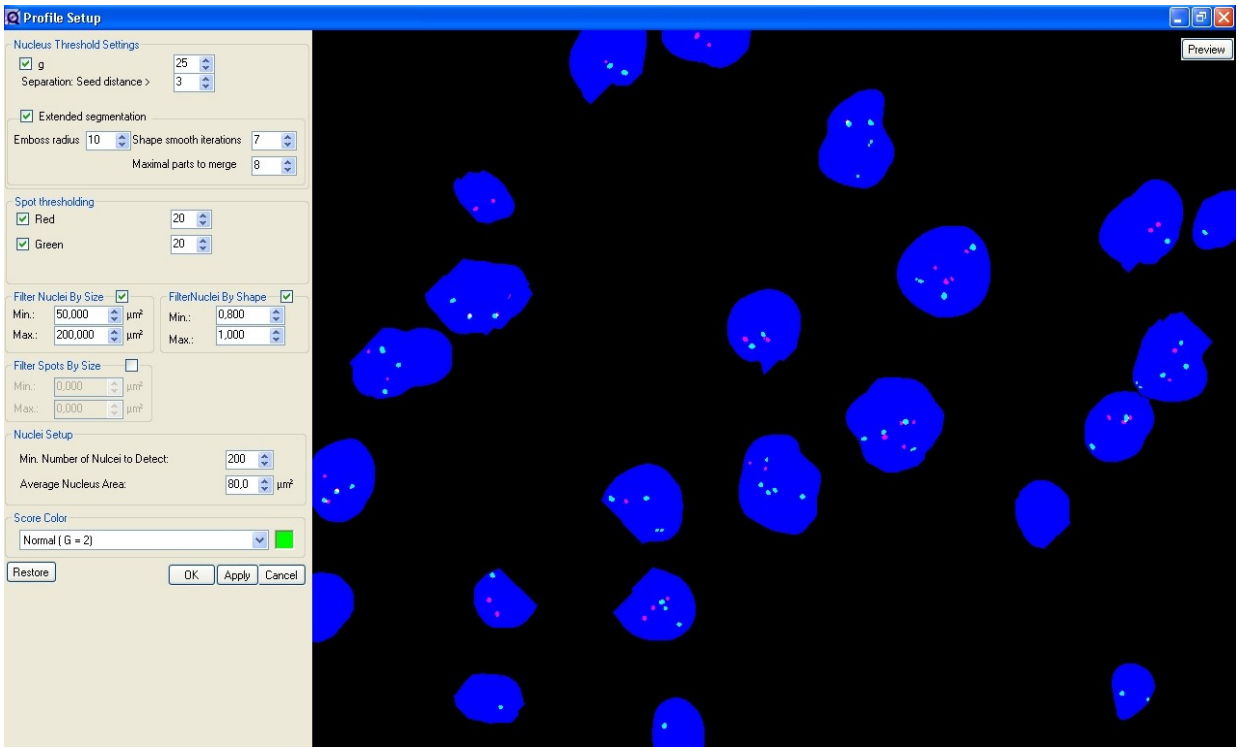


FISH Settings	Setting or changing **Profile Setup** of the FISH algorithm.
Channel Settings	Specifying BRG color attributes .
Annotation Selection	Selecting *annotation*(s) for the measuring process.
Process Annotation Area	The algorithm will be executed only on the selected *annotation*s.
Process Whole Slide	The algorithm will be executed on the whole *digital slide* area.
Export settings	The *measurement*s will be exported to a *CSV* file that can be opened and edited further in Microsoft Excel.

Important!

FISHQuant is able to work with slides scanned in **appropriate channel order (B-G-R) and with adequate channel name (blue/DAPI/Aqua; green/FITC; red/orange/rhodamine) only.**

6. Click **ProfileSetup** and set the following attributes:



Nucleus Threshold settings	Specifying the limit values in DAPI channel; enabling Hole Filling with the DAPI mask if necessary.
Extended Segmentation	Especially for tissue samples. Emboss radius (1-100), Shape smooth iteration (1-100) and Maximal parts to merge (2-16) can be defined.

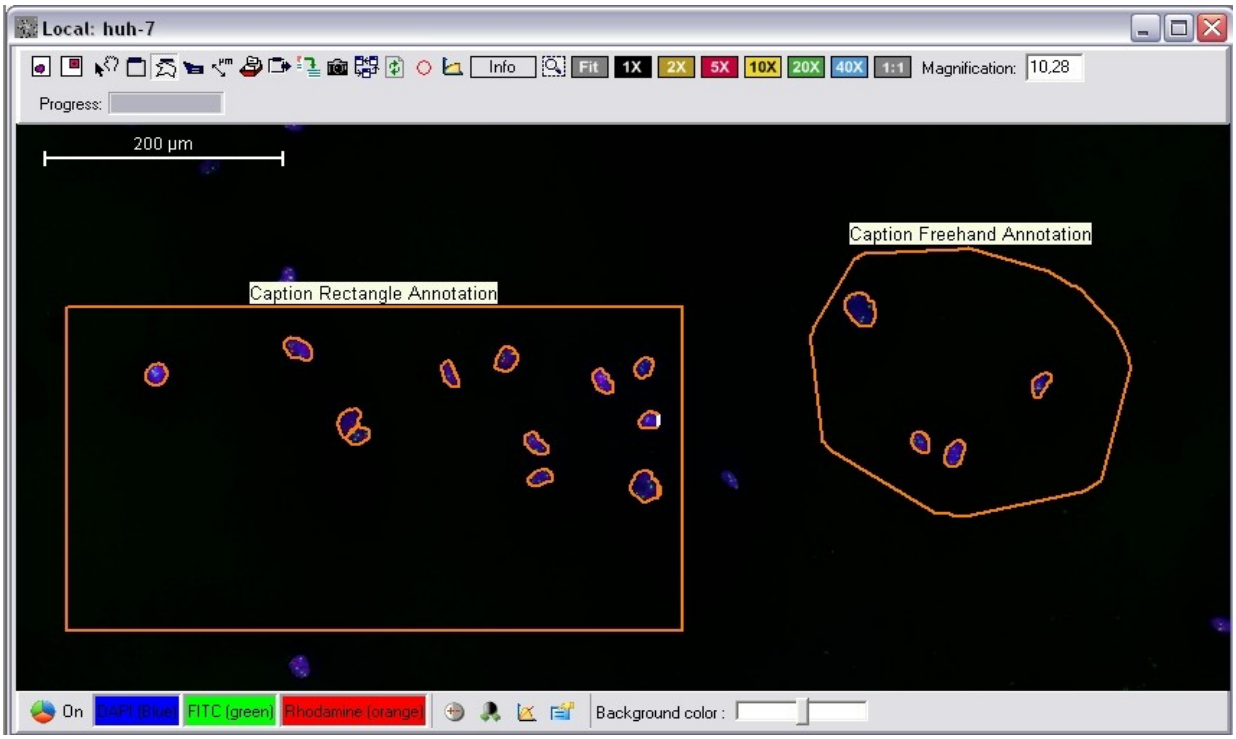
Spot Thresholding	Specifying the limit values in FITC and Rhodamine channels.
Nucleus filtering	Nuclei can be filtered based on their shape factor and size.
Spots filtering	Spots can be filtered by size.
Nuclei setup	Setting the number of nuclei to measure and the average nucleus area for segmentation.
Score Color	Score-specific labelling can be defined.

7. Click **OK** to save settings and the form will be closed.

Save Region measurements	The *measurements* will be exported to a *CSV* file that can be opened and edited further in Microsoft Excel.
Save Cumulative measurements	The *measurements* will be exported to *CSV* files. A cumulative file will be created for each detected *layer*.
Save to single CSV	The *measurements* will be exported to a single *CSV* file. This file will contain all the parameters of the *layers*.

8. Launch the FISH Analysis algorithm by clicking **Start**.

   The process is fully automated, you only need to click **Close** when FISH Analysis has finished. FISH Analysis leaves open the slide. If more than one slide is open when you start **FISHQuant**, **FISHQuant** starts using the active one.



Tip!

To view the existing *annotation*s and existing *measurement*s on the slide, click INFO in the slide toolbar and then select **Show Annotations** or **Show HQ Measurements** from the shortcut menu.

3.4 Running Batch Process in FISHQuant

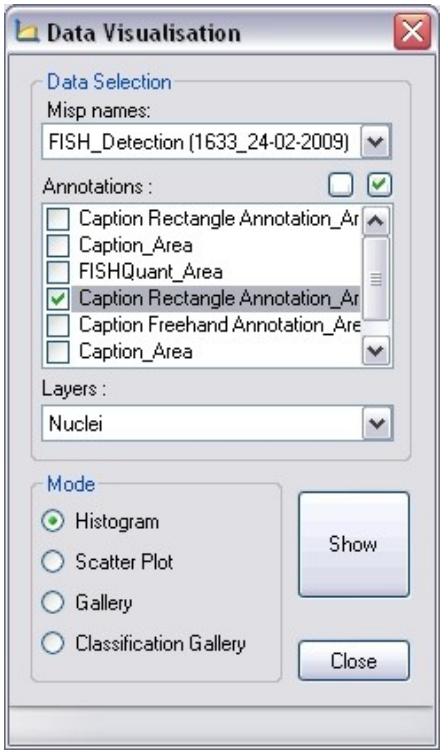
In **Image Analysis Modules** menu, you have the option to work with batch files. Select the slides you want **FISHQuant** to work with and specify the parameters for which they should be analyzed. Every slide needs to have the **correct channel name and order**, as mentioned above.

4 Working with Measurement Visualization

The **Measurement Visualization** module for MIRAX Viewer creates a scatter plot or a histogram from the **FISHQuant** *measurement*s.

4.1 Creating a Histogram

1. Click in the slide toolbar in the MIRAX Viewer.



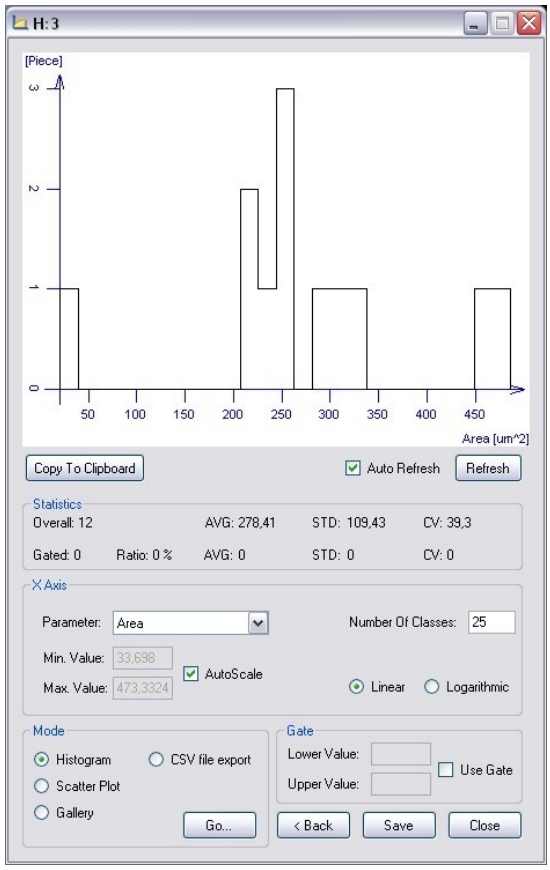
2. Select the name of *measurement* from the drop-down menu that has been previously saved to the slide.

3. Select the *annotation*s that you want to analyze. You can select all by checking the **Select all** option.

4. Select the *object layer* that you want to display from the **Layer** drop-down menu.

5. Select **Histogram** and the press **Show** button**.**

6. Set the parameters. If you enter new values into the text *field*s, press **Enter** to refresh the diagram.



Auto Refresh	Automatically updates the histogram when you change the settings for the parent form.
Statistics	Overall shows the quantitative data of the *objects*.
X Axis	Offers various parameters for the X axis. When you export the results into a *CSV* file, there is an explanation for the abbreviations of the parameter names.
Gate	Chooses an interval within the examined range on X axis.
Mode	By using Gate function, the program either creates new histogram, new scatter plot, shows gallery or opens a new form where the user can save *CSV* files, but only in the selected interval. By not using Gate function, the program uses all the above mentioned features but for the whole data range.

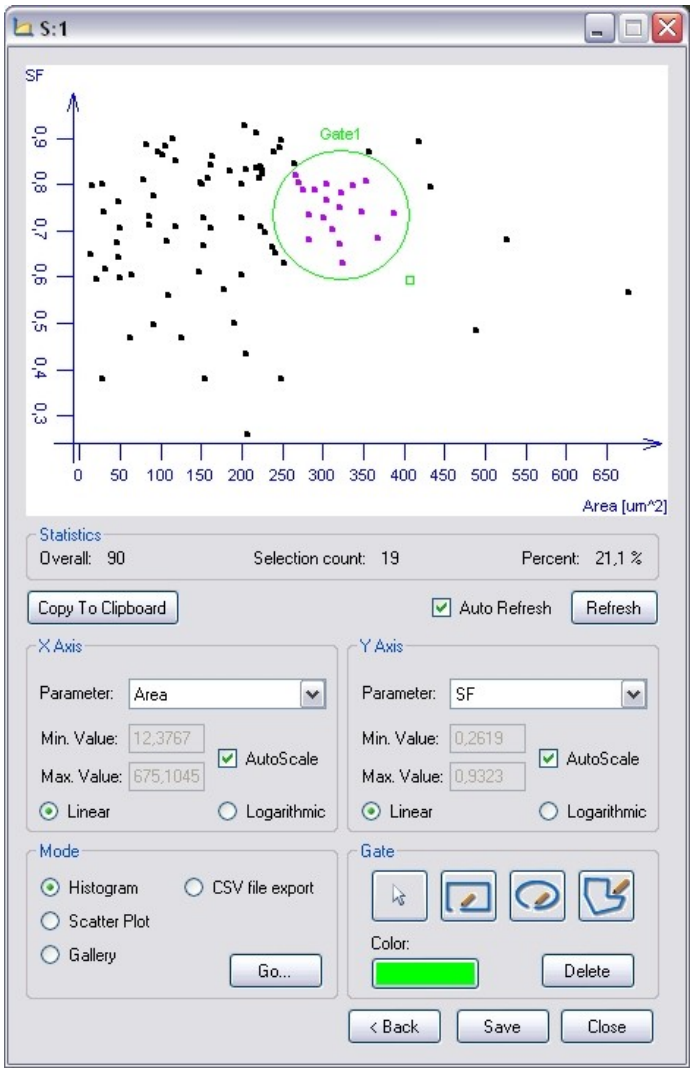
As the measurements run on digital slides, the perimeter and area sizes of the very small objects (appropriating the pixel-size) can show some inaccuracy.

7. [optional]

   To create a snapshot of the histogram, click **Save**.

4.2 Creating a Scatterplot

1. Click in the slide toolbar in the MIRAX Viewer.
2. Select the name of *measurement*, from the drop-down menu, that has been previously saved to the slide.
3. Select the *annotation*s that you want to analyze.
4. Select the *object layer* that you want to display.
5. Select **Scatter Plot** and the press the **Show** button.
6. Set the parameters. If you enter new values into the text *field*s, press **Enter** to refresh the diagram.



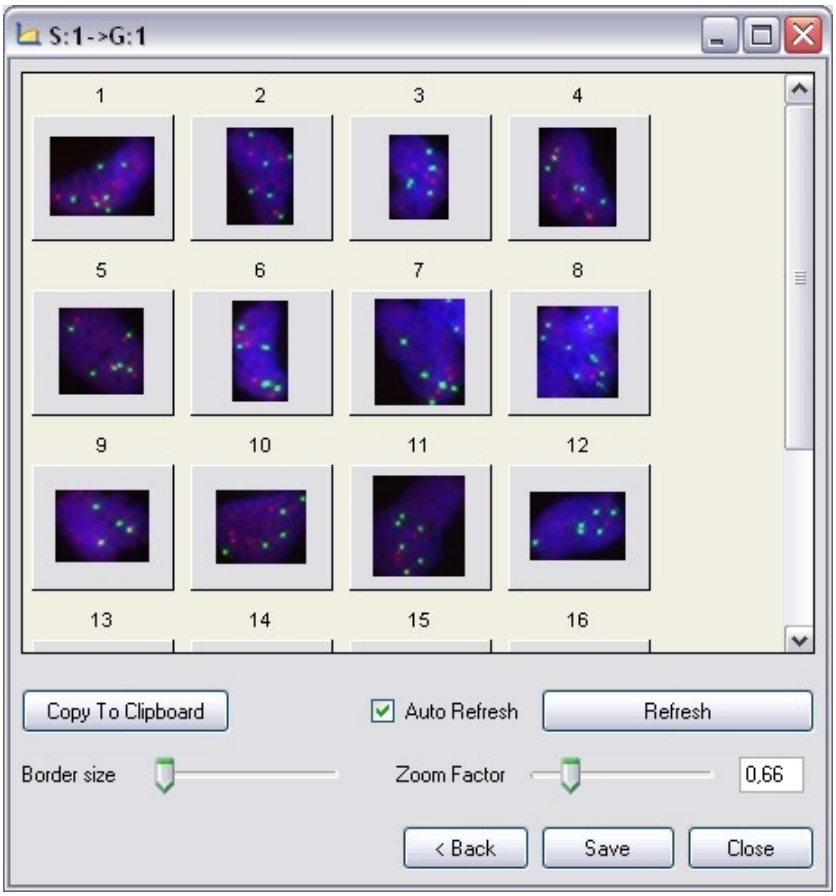
Auto Refresh	Automatically updates the histogram when you change the settings for the parent form.
Statistics	Overall it shows the quantitative data of the *objects*.
X Axis	Offers various parameters for the X axis.
Y Axis	Offers various parameters for the Y axis.
Gate	Chooses an interval within the examined range on X axis. You can position the scatter plot gates by clicking on the arrow.
Mode	By using Gate function, the program either creates new histogram, new scatter plot, shows gallery or opens a new form where the user can save *CSV* files, but only in the selected interval. By not using Gate function, the program uses all the above mentioned features, but for the whole data range.

7. [optional]

   To create a snapshot of the scatterplot, click **Save**.

4.3 Creating a Gallery

1. Click on the slide toolbar in the MIRAX Viewer.
2. Select the name of the *measurement*, from the drop-down menu, that has been previously saved to the slide.
3. Select the *annotation*s that you want to analyze.
4. Select the *object layer* that you want to display.
5. Select **Gallery** and the press the **Show** button**.**
6. Set the parameters.



Auto Refresh	Automatically updates the histogram when you change the settings for the parent form.
Border size	Sets the size of the image border.
Zoom factor	Sets the zooming rate of the images. You can determine the zooming value by entering the exact rate in the text *field*.

Moving the cursor over an image an info box appears conveying all the necessary data regarding that image.

7. [optional]

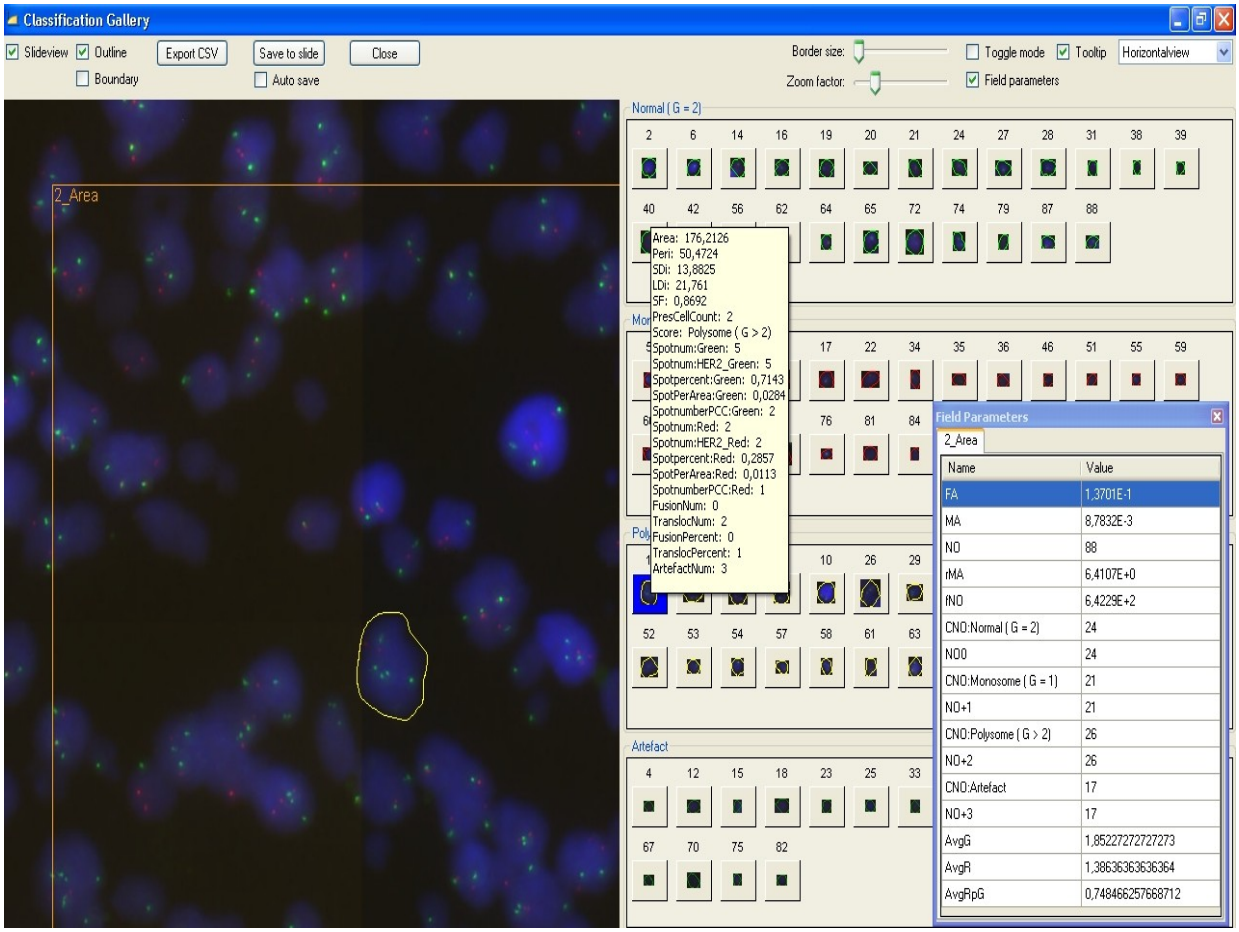
   To export the gallery as an image, click **Save**.

Tip!

If you select an image in the gallery, it will be highlighted in the Scatter Plot as well.

4.4 Creating a Classification Gallery

You can group your *objects* in accordance with their score values (the probe used on the *objects*). You can revalidate and **rescore** your *object*. On the right side panel, you can move cell nuclei from one group to the other. On the left panel, you can view the selected cell nuclei.



Slideview	Shows the selected slide.
Outline	Shows the group-specific outline.
Boundary	Shows the number of the detected *objects*.
Export CSV	Exports the *measurements* to a *CSV* file.
Save to slide	Saves the changes to the slides.
Auto save	Automatically saves all changes. Note, that it decelerates the program.
Border size glide	Increases / decreases the size of *object* borders in the right panel.
Zoom factor glide	Zooms in / out on the *objects* in the right panel.

Toggle mode	You can select *object*s with the help of your mouse in the right panel. You can rescore *objects* using right-click.
Field Parameters	Shows the field specific parameters.
Tooltip	Moving cursor over an *object* shows the basic information of the given *object*.
View	You can select among normal, horizontal or vertical view of the *object*s in the right panel.

Related documents

MIRAX Viewer 1.12 User's Guide

ImmunoScreener Module for MIRAX Viewer 1.12, User's Guide

MIRAX SCAN User's Guide

http://books.google.com/books?id=kvWKgG6iPXMC&dq

Index

C
computation time.....9
L
Layer.....5
M
MISP.....5
S
Segmentation.....5
Segmentation Profile.....5

Appendix A FISH Analysis

A.1 Object-specific parameters

Parameter name	Description
Area	Area of the detected *object* in µm^2
BInt	B intensity of the detected *object*
GInt	G intensity of the detected *object*
GrayInt	Gray intensity of the detected *object*
IF_C1	Integrated fluorescence in channel 1
IF_C2	Integrated fluorescence in channel 2
IF_C3	Integrated fluorescence in channel 3
LDia	Longest diameter of the detected *object* in µm
Peri	Perimeter of the detected *object* in µm
RInt	R intensity of the detected *object*
SDi	Shortest diameter of the detected *object* in µm
SF	Shape factor of the detected *object*

A.2 Summing parameters for an area

Parameter name	Description
AvgPos0	Average positivity of negative Nuclei
AvgPos+1	Average positivity of +1 Nuclei
AvgPos+2	Average positivity of +2 Nuclei
AvgPos+3	Average positivity of +3 Nuclei
FA	Overall *annotation* area in mm^2
fNO	NO/FA; frequency of detected *object*s per µm^2
HSCORE	
MA	Overall mask area in mm^2: Total area of all detected *object*s in each *layer*.
MA0	Mask area of negative Nuclei
MA+1	Mask area of +1 Nuclei
MA+2	Mask area of +2 Nuclei
MA+3	Mask area of +3 Nuclei
NO	Number of detected *object*s: Total number of each detected *object* in each *layer*
NO0	Number of negative Nuclei
NO+1	Number of +1 Nuclei
NO+2	Number of +2 Nuclei

NO+3	Number of +3 Nuclei
rMA	(MA/FA)*100; relative mask (*object*) area in %
rMAPos	(('MAO+1' + 'MAO+2' + 'MA+3') / MA)*100
rNOPos	(('NO+1' + 'NO+2' + 'NO+3') / NO)*100